American Journal of Medical Genetics 64:293–295 (1996)

# Deletion in the FMR1 Gene in a Fragile-X Male

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**The pathogenesis of Fragile-X syndrome is a consequence of absence of the FMR1 gene product associated with expansion of the CGG repeat and abnormal methylation of this and a CpG island 250 bp proximal to the CGG repeat located at exon 1 in the FMR1 gene. While this is usually the case, some suspected Fragile-X syndrome patients have been described with a mutation other than CGG expansion. We describe here an affected Fragile-X male, who was found to be mosaic of a full mutation of the CGG expansion and a deletion in the FMR1 gene. The patient's phenotype is probably mainly due to the effect of the full mutation of the repeat sequence. Thus, the influence of the deletion is difficult to evaluate.**


**KEY WORDS: mental retardation, deletion, mosaicism**

## INTRODUCTION

Fragile-X syndrome is one of the most common forms of mental retardation, with a frequency of 1 to 1,500 males and 1 to 2,500 females [Webb et al., 1986], and manifestations of severe mental retardation, developmental delay, and such physical characteristics as large ears, long face with a prominent jaw, and macroorchidism. This syndrome is inherited as an X-linked dominant disorder with reduced penetrance. Fragile-X syndrome is associated with a folate-sensitive fragile site, FRAXA, at Xq27.3 [Lubs, 1969].

The pathogenesis of Fragile-X syndrome is a consequence of absence of the FMR1 gene product associated with expansion and abnormal methylation of the CGG repeat located at exon 1 in the FMR1 gene [Kremer et al., 1991; Oberlé et al., 1991; Verkerk et al., 1991; Yu et al., 1991]. While this is usually the case, some suspected Fragile-X syndrome patients have been described with a mutation other than CGG expansion. Large deletions involving removal of the whole FMR1 gene have been reported [Gedeon et al., 1992; Tarleton et al., 1993; Gu et al., 1994; Quan et al., 1995] but shorter deletions have also been found, where absence of FMR1-expression can be explained by loss of sequences proximal to the FMR1 gene and/or sequences flanking and including the CGG repeat region [Wohrle et al., 1992; Meijer et al., 1994; Trottier et al., 1994; Hirst et al., 1995]. In addition, de Boulle et al. [1993] have described a point mutation resulting in a $367^{Ile} \rightarrow 367^{Asn}$ substitution in the FMR1 gene and leading to the Fragile-X phenotype.

We describe here a detailed sequence analysis of the FMR1 gene of an affected Fragile-X male, who was found to be mosaic for a full mutation of the CGG expansion and a deletion in the 2.8 kb *EcoR*I-*Eag*I fragment recognized by the StB12.3 probe.

## MATERIALS AND METHODS

### Case Presentation

The index case is a 52-year-old Finnish man with moderate mental retardation, with no ability to read or write, now attending a sheltered workshop. He has a classic Fragile-X phenotype with obesity. Of the family, only a phenotypically healthy sister is alive.

### DNA Analysis

The probe StB12.3 [Oberlé et al., 1991] was used for Southern blot analysis of the FMR1 gene. PCR analysis of the deleted area was carried out as described by Chong et al. [1994] with the primers 5′-GGAACAGCGTTGATCACGTGACGTGGTTTC-3′ and 5′-GGGGCCTGCCCTAGSGCCAAGTACCTTGT-3′. The PCR product was subjected to direct sequencing utilizing a USB PCR product sequencing kit, with $^{35}$S-dATP as an isotope, according to the manufacturer's instructions.

## RESULTS AND DISCUSSION

Although most Fragile-X patients have an expanded CGG repeat sequence in the FMR1 gene, it is now becoming evident that other molecular mechanisms also exist leading to an affected phenotype. A full mutation with over 200 CGG repeats represses FMR1 transcription [Pieretti et al., 1991; Verheij et al., 1993] and results in reduction of the amount of translated FMR1 protein. In this study, analysis of the index case demon-

Received for publication September 13, 1995; revision received December 21, 1995.

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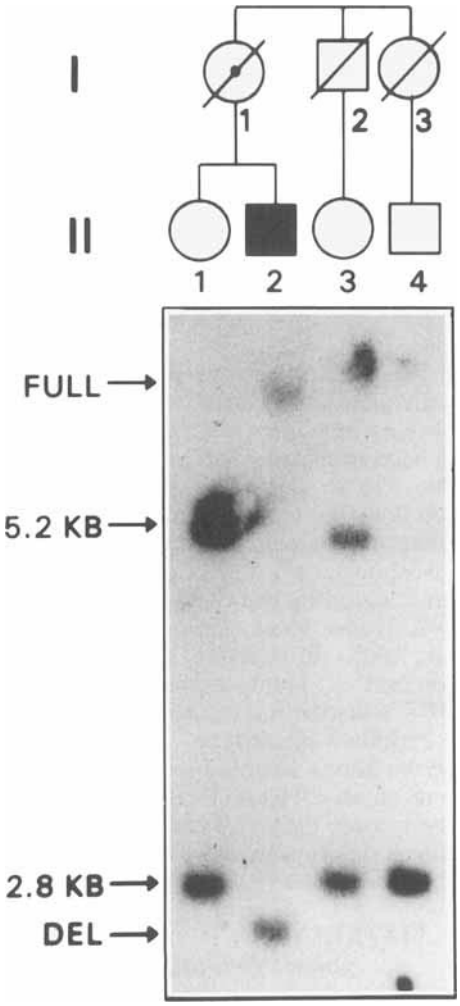


Fig. 1. Southern blot showing the CGG repeat of the FMR1 of a fragile X male (II-2) and his relatives. DNA obtained from peripheral blood was digested with *EcoR*I and *Eag*I and hybridized with StB12.3. The specimen of the proband shows a mosaic situation with a smear of full mutation size and an abnormally small fragment.

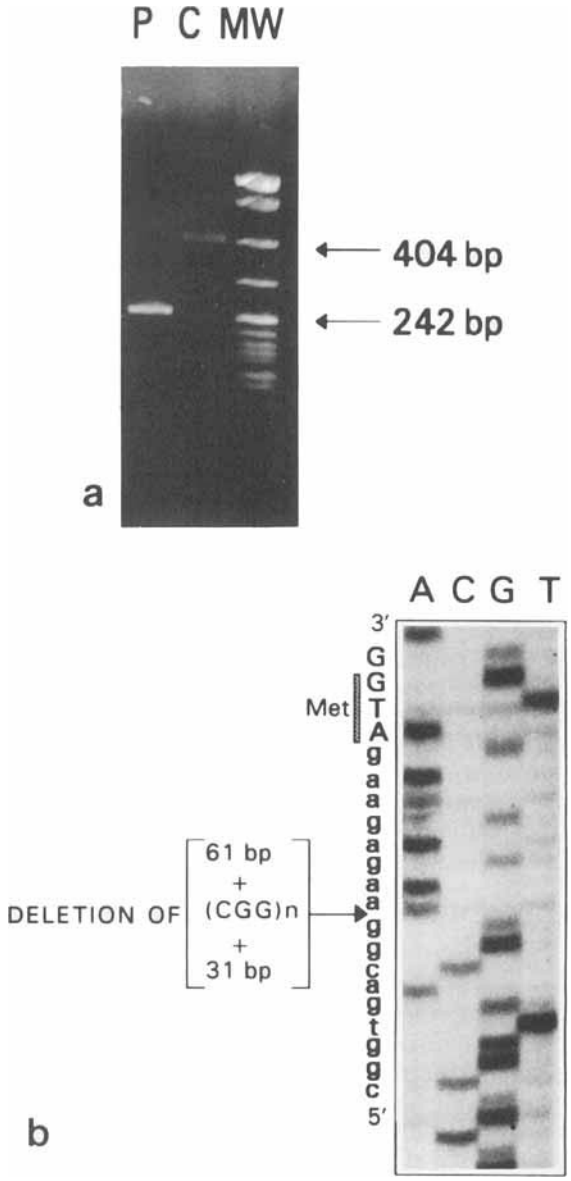


Fig. 2. PCR amplification as described by Chong et al. [1994] and sequencing of the deleted area. **a:** Amplified PCR-products were sized in an agarose gel electrophoresis and stained with ethidium bromide. P, patient; C, control sample; MW*Msp*I digested pBR322 as a molecular weight marker. **b:** The sequenced PCR product from the untranslating region of the FMR1 gene of the proband demonstrates a deletion spanning the entire CGG repeat and flanking sequences of 31 bp and 61 bp.

strated a mosaic banding pattern with a full methylated mutation in the CGG region, and a shortened *EcoR*I-*Eag*I fragment compared with the 2.8 kb normal sized fragment (Fig. 1).

To further assess the size and location of the deletion in the FMR1 gene, PCR-based analysis (Fig. 2a) and direct sequencing was utilized. A deletion spanning the entire CGG-repeat and flanking sequences of 31 bp at the 5′-side and 61 bp at the 3′-side of the CGG-repeat within the untranslating region was found (Fig. 2b).

The patient described in this paper presents a rare type of mosaicism in Fragile-X males. Similar cases with both a full methylated mutation and a deletion in and adjacent to the CGG repeat sequence have previously been reported by de Graaf et al. [1995] and De Vries et al. [1993]. The deletion was found not to disrupt the coding sequence of the FMR1 gene. Although this alteration is located at the hotspot deletion region described by de Graaff et al. [1995], the influence of this deletion on FMR1 gene expression and protein synthesis is difficult to interpret, since the promoter region of the FMR1 gene is only partially described. Furthermore, the phenotypic effect of this deletion is probably obscured by the effect of the full mutation of the CGG repeat. It is also possible that the deletion is a consequence of the instability of the full mutation sized CGG repeat [de Graaff et al., 1995]. Additional information concerning the impact of the deletion described here could be gained through in vitro gene expression studies.

## ACKNOWLEDGMENTS

We thank Dr. Salme Majuri for referring the patient and Mrs. Pirkko Jokela and Mrs. Inkeri Happonen for carrying out the Southern hybridization. We also thank the proband and his relatives for their cooperation.

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